# Freshwater Beach
# Total Maximum Daily Load
# Microbial Source Tracking Study

A final report to the
New Hampshire Department of Environmental Services

Submitted by

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# Introduction

This study compliments TMDLs that were conducted simultaneously by the New Hampshire Department of Environmental Services (NHDES). Each season DES posts bacteria advisories for at least ten designated beaches. The overall project goal was to allow NHDES to develop pathogen TMDLs and beach management plans for source specific bacterial loads at public beaches statewide. Microbial source tracking was used to identify non-point source species of bacteria at the study beaches to help determine load reductions needed to meet state standards. The development of the beach TMDLs will allow the state to produce a TMDL for every freshwater beach listed as impaired. DES will use the results of this study to produce a final status report and beach management plan. DES will produce a draft TMDL by June 2006.

# Project Setting

The TMDLs were at three impaired assessment units (AUs): Sand Dam Village Pond Town Beach (NHIMP802010303-04-02) in Troy, NH, Pawtuckaway Lake-Pawtuckaway State Park (NHLAK600030704-02-02) in Nottingham, NH, and Mill Pond Town Beach (NHIMP700030204-05-02) in East Washington, NH (See Appendix A for beach specific maps). These assessment units were listed as impaired in the Consolidated Assessment and Listing Methodology (CALM) for primary contact recreation. The pollutant of concern was *Escherichia coli* (*E. coli*). The suspected sources of *E. coli* at each beach area were as follows: Canada Geese at Sand Dam Village Pond Town Beach, bather loads at Pawtuckaway Lake-Pawtuckaway State Park, and agriculture at Mill Pond Town Beach.

# Project Goals and Objectives

The goal of this project was to investigate actual and potential bacterial sources at (3) public beaches. The approach reflects the latest concepts for efficient use of bacterial ribotyping for pollution source identification in New Hampshire, i.e., ribotyping of high priority samples and development of small local source species databases. This targeted approach was designed to optimize identification of the most significant contamination sources at the 3 beaches. The specific objectives were to:

1. Isolate and ribotype strains of *E. coli* from scat samples collected in the areas surrounding the three target beaches for construction of a local database of ribopatterns for this study.

2. Isolate strains of *E. coli* from water samples collected at the three target beaches.

3. Ribotype strains of *E. coli* isolated from water samples considered high priority by NHDES.

4. Compare ribopatterns from water samples with the local and New Hampshire source species databases to identify sources of bacteria at the targeted public beaches.

5. Write a final report including an analysis and interpretation of the riboptying data.

# Methods

## Sample Locations and Timing

The Beach Program conducted dry and wet weather sampling at three public beaches (Table 1) previously identified as having bacterial pollution problems:

-Pawtuckaway State Park is located in Nottingham, NH on the southern end of Pawtuckaway Lake. The beach is located along the shores of Pawtuckaway Lake in the 5,500 acre park. The beach is a popular recreation area for campers and the public with an onsite bathhouse. The surrounding area is heavily forested and frequented by wildlife. The beach experiences a heavy bather load during the summer months with over 80,000 visitors a year. Canada geese and ducks are often found using the beach area. (See Appendix A for a detailed map)

-Sand Dam Village Pond Town Beach is located in Troy, NH. The beach area is surrounded by a town park with a bathroom, tennis courts and ball field; the rest of the area is light residential. The beach and park are home to a large population of Canada geese from spring through the fall. (See Appendix A for a detailed map)

-Mill Pond Town Beach is located in East Washington, NH. The beach is located on an impoundment that drains a rural, agricultural area. The majority of residences in the area are home to a variety of livestock including horses, cows, chickens, goats, sheep and even emus. (See Appendix A for a detailed map)

**Table 1. Sampling location at three freshwater beaches in New Hampshire.**

| Beach location | Beach name | Assessment unit | Sample site designation | Sample area on beach |
|---|---|---|---|---|
| Nottingham | Pawtuckaway State Park Beach | NHLAK600030704-02-02 | NOTLF | Left |
| | | | NOTCR | Center |
| | | | NOTRT | Right |
| Troy | Sand Dam Village Pond Town Beach | NHIMP802010303-04-02 | TROLF | Left |
| | | | TROCR | Center |
| East Washington | Mill Pond Town Beach | NHIMP700030204-05-02 | WASLF | Left |
| | | | WASRT | Right |

There were at least two sampling locations (left-LT, center-CR, right-RT) at each beach, as noted in Table 1. All but the last sample were collected during the beach season (June to Labor Day). The samples were collected during dry weather on 8 occasions (7/5, 7/6, 7/21, 8/3, 8/4, 8/18, 8/30, 9/21) and during 2 wet weather events (8/15, 8/29). Wet weather events were defined as days with > 0.25 inches of rain in the previous 24 hours. On 8/3/05 there was > 0.25 inches of rain in the previous 48 hours. Five sample days (7/5, 7/6, 7/21, 8/18, 8/30) had > 0.01 but < 0.25 inches of rainfall prior to sampling.

Scat samples were collected from suspected pollution sources in close proximity to the three beaches. Water and scat samples were collected by NHDES personnel and delivered to JEL the same day. The samples were stored at 4-7 °C until analysis, which was initiated within 2 hours of receipt of the samples.

## Laboratory and Analytical Methods

### *Detection and Identification of Fecal Coliforms and E. coli*

Appropriate volumes of water samples were filtered to give at least 20 colonies on agar plates, where possible. The membrane filters were rolled onto mTEC agar in Petri dishes. Plates were inverted and incubated at 44.5±0.2 °C for 24 hours (USEPA, 1986). Fecal coliforms were enumerated by counting the yellow colonies after the incubation period, and *E. coli* was enumerated by counting the yellow colonies on the plate following incubation of the filter on urea substrate (Jones and Bryant 2002, Rippey et al. 1987).

Following urease testing, each plate was inspected and the plate giving countable (20-60) colonies was used for selection of individual *E. coli* strains for analysis. For some samples, fewer than 20 colonies were present on the smallest dilution analyzed, so the plate with the most numerous colonies was used. The *E. coli* isolates were subject to a

battery of biochemical tests to confirm their identity as *E. coli*. The procedures used for isolating and identifying *E. coli* strains for this study were according to standard lab protocols (Landry 2004, Jones 2002a, Jones and Bryant 2002). The confirmed *E. coli* isolates were then processed for determining ribopatterns.

***Sample Processing***

The procedures used for ribotyping *E. coli* isolates for this study have been used previously (Jones et al. 2004 a&b, Jones and Landry 2003, Jones, 2002b) and are based to a large extent on those of Parveen et al. (1999). *E. coli* isolates were stored in cryovials at -80°C and re-cultured onto trypticase soya agar (TSA). Some of the stored isolates could not be re-cultured. Cultures on TSA were incubated overnight at room temperature (~20°C). Some of the resulting culture was transferred to duplicate cryovials containing fresh glycerol/DMSO cryo-protectant media for long-term storage at -80°C.

A RiboPrinter® was used to process *E. coli* culture for ribotype determinations. After preparation of the samples, the automated process involved lysing cells and cutting the released DNA into fragments via the restriction enzyme EcoR1. These fragments were separated by size through gel electrophoresis and then transferred to a membrane, where they were hybridized with a DNA probe and mixed with a chemiluminescent agent. The DNA probe targeted 5S, 16S and 23S ribosomal RNA genes. A digitizing camera captured the light emission as image data, from which the system extracted a RiboPrint® pattern. This pattern could be compared to others in the RiboPrinter® database for characterization and identification based on densiometry data, although our approach has conformed to other ribotyping studies in using banding patterns as the basis for comparing patterns.

***Band Pattern Identification***

The images were transferred from the RiboPrinter® into GelComparII (Applied-Maths) analytical software. The bands in lanes containing the standard were labeled and entered into the memory for optimization of gel pattern images. The densiometry data were processed for band identification using a minimum threshold for band detection of 1%. The ribopattern data for each separate water sample isolate were then selected for identification of source species.

***Source Species Database***

The analysis of the water isolate ribopatterns for identification of source species was based initially on a local source species database from the study sites and then on a New Hampshire source species database (Table 2). The local database for the beach study areas contained ribopatterns from each of 10 scat samples from 3 geese, 1 septage, 1 sheep, 2 horse, 1 cow, 1 duck and 1 goat. There were 20 *E. coli* strains isolated from each sample, from which 6 were ribotyped. The New Hampshire database contained 735 unique ribotypes from 33 different source species, including wastewater, septage and direct human sources (Table 2).

**Table 2. Source species databases for New Hampshire and this study.**

| Species | NH database | Local database |
|---|---|---|
| Alpaca | 2 | |
| Beaver | 7 | |
| Buffalo | 8 | |
| Cat | 7 | |
| Chicken | 19 | |
| Cormorant | 10 | |
| Cow | 50 | 6 |
| Coyote | 25 | |
| Deer | 75 | |
| Dog | 36 | |
| Duck | 14 | 4 |
| Goat | 8 | 4 |
| Goose | 60 | 17 |
| Horse | 45 | 10 |
| Human | 30 | |
| Mouse | 2 | |
| Muskrat | 6 | |
| Otter | 9 | |
| Oxen | 4 | |
| Pigeon | 4 | |
| Rabbit | 24 | |
| Racoon | 61 | |
| Red Fox | 32 | |
| Robin | 2 | |
| Seagull | 25 | |
| Septage | 14 | 6 |
| Sheep | 5 | 4 |
| Skunk | 4 | |
| Sparrow | 3 | |
| Starling | 1 | |
| Unidentified Wildlife | 17 | |
| Wastewater | 121 | |
| Wild Turkey | 5 | |
| **Totals** | **735** | **51** |

***Data Analysis***

All ribotyping data were analyzed with GelComparII software on a Dell computer. Hard copies of ribotype patterns and similarity coefficients for each unknown water isolate and its most closely related source species were printed for interpretation. Interpretation and accompanying graphical representations of the data were done using MS Excel on Macintosh computers.

Optimization was set at 1.50% and band position tolerance was set at 1.00%. Both of these parameters relate to the ability to differentiate between bands for the degree of accuracy desired, and also to compensate for possible misalignment of homologous bands caused by technical problems. Tolerance and optimization settings can be modified to influence the similarity coefficient used and result in a greater number of identified

source species. However, a balance is required between stringency of data analysis parameters, the fraction of isolates that can be identified and consistency of methods between studies. The use of a QA *E. coli* strain (ATCC #51739) in the analysis for this study and comparison to past analyses of this strain gave acceptable (90%) matching of resulting ribopatterns.

Similarity indices between sample and database ribopatterns were determined using Dice's coincidence index (Dice, 1945) and the distance among clusters calculated using cluster analysis. The source species profile with the highest similarity coefficient was accepted as an indication of the possible source species for the water sample isolate. For this study, the predetermined threshold similarity index that was considered to be a minimum value for identifying source species was 90%. If the value calculated for a water isolate was below the threshold similarity index, the water sample isolate was considered to be of unknown origin.

Cluster analyses were performed to determine the relationships among isolates from the same source species and the same sites, and to identify banding patterns that were identical for different isolates. The cluster analyses were based on the un-weighted pair group method by arithmetic averaging (UPGMA) or the neighbor joining algorithms. The last step in data analysis was visual inspection of the band matching results. Hard copies of ribotype patterns and similarity coefficients for the unknown and most closely related source species were printed for verification of statistical analyses and further interpretation. Data analysis and accompanying tabular representations of the data were done using MS Excel on Macintosh computers.

# Results and Discussion

## Sample Frequency and Locations

Water samples were collected one to three times at each of the three Nottingham sites, three times each at the two Troy sites and two to four times at each of the two East Washington sites (Table 3). The samples containing the highest *E. coli* concentrations were selected for ribotyping.

**Table 3. Ribotyping summary for *E. coli* isolates for water samples collected from freshwater beaches: 2005.**

| Sample location | Total # samples | Total # isolates | # samples ribotyped | # isolates ribotyped |
|---|---|---|---|---|
| **NOTLF** | 3 | 15 | 2 | 10 |
| **NOTCR** | 2 | 10 | 1 | 5 |
| **NOTRT** | 1 | 5 | 1 | 5 |
| **TROLF** | 3 | 15 | 3 | 15 |
| **TROCR** | 3 | 15 | 2 | 10 |
| **WASLF** | 2 | 10 | 2 | 10 |
| **WASRT** | 4 | 20 | 4 | 20 |
| Totals | 18 | 90 | 15 | 75 |

## Bacteria Concentrations at the Three Beaches

Fecal coliform and *E. coli* (FC/EC) concentrations in beach water samples were measured (Table 4). Concentrations ranged from 16 to 8,400 FC/100 ml and from 12 to 8,000 *E. coli*/100 ml. The FC:*E. coli* ratios for all samples were relatively high (>74%). The limit for posting beach advisories is 88 *E. coli*/100 ml, and half (9) of the samples exceeded this limit. Five of the six samples from the East Washington beach exceeded the limit while only two of the six samples from each of the other two beaches exceeded the limit. Relatively high *E. coli* concentrations (>6400 cfu/100 ml) were measured in one sample each from the Nottingham and East Washington beaches. The *E. coli* concentrations in the two samples collected during wet weather events were not significantly different than concentrations measured in dry weather samples.

**Table 4. Fecal coliform/*E. coli* concentrations (cfu/100 ml) for water samples collected from freshwater beaches: 2005.**

| Site | **7/5/05** | **7/6/05** | **7/21/05** | **8/3/05** | **8/4/05** | **8/15/05** | **8/18/05** | **8/29/05** | **8/30/05** | **9/21/05** |
|---|---|---|---|---|---|---|---|---|---|---|
| **NOTLF** | - | - | 48/40 | - | 16/12 | 56/52 | - | - | - | - |
| **NOTCR** | - | 8400/8000 | - | - | - | - | - | - | - | 36/36 |
| **NOTRT** | - | - | - | - | - | - | - | 146/144 | - | - |
| **TROLF** | - | - | 168/128 | 36/36 | - | - | - | - | 74/72 | - |
| **TROCR** | 68/68 | - | - | - | - | - | 420/420 | - | - | 28/28 |
| **WASLF** | - | - | 172/128 | 7200/6400 | - | - | - | - | - | - |
| **WASRT** | - | - | 108/92 | - | - | - | 216/196 | - | 412/398 | 80/80 |

Highlighted cells indicate samples that were not ribotyped

The basis for choosing samples for ribotyping was based on *E. coli* concentrations, where samples with the highest ($\geq$ 68/100 ml) concentrations were selected and samples with the lowest ($\leq$36/100 ml) concentrations were not ribotyped. One sample with an *E. coli* concentration of 36/100 ml was included in the ribotyping because the isolates had already been ribotyped prior to final decisions on which samples

to ribotype. Isolates from only three water samples were not included for ribotyping, and a total of 75 isolates from water samples were ribotyped for source species identification.

## Local Scat Samples and Source Species Database

Scat samples from the beach study areas included those from 3 geese, 1 septage, 1 sheep, 2 horse, 1 cow, 1 duck and 1 goat (Table 5). The *E. coli* concentrations (per g wet weight) ranged from >2 x $10^8$ for all 3 geese samples to < 1000 for septage. The order for *E. coli* concentrations in descending order was geese >> sheep > horse >> goat > cow>> duck > horse > sepatage. There were 20 *E. coli* strains isolated from each sample. Support for analytical costs was available for ribotyping 6 isolates from each scat sample, providing a total of 60 local ribotypes to be used for identifying source species. The ribopatterns contained 7-15 bands. Some of the resulting ribopatterns were identical amongst isolates from the same sample. These duplicate patterns were excluded from the database. The final number of unique patterns was 51 (85% of total), and these are summarized for each source species sample in Table 5.

**Table 5. Ribotyping summary for *E. coli* isolates from scat samples collected from freshwater beaches.**

| Sample | Species | Date | Location | *E.coli* Concentration | # Colonies Speciated | # *E.coli* Confirmed | # Isolates Ribotyped | # of Ribopatterns |
|---|---|---|---|---|---|---|---|---|
| | | | | cfu/g wet wt. | | | | |
| GE1 | Geese | 7/21/05 | Troy | >222,000,000 | 20 | 6 | 6 | 5 |
| GE2 | Geese | 7/21/05 | Troy | >222,000,000 | 20 | 6 | 6 | 6 |
| GE3 | Geese | 7/21/05 | Nottingham | >222,000,000 | 20 | 6 | 6 | 6 |
| ST1 | Septage | 9/6/05 | Nottingham | 789 | 20 | 6 | 6 | 6 |
| SP1 | Sheep | 10/3/05 | East Washington | 5,888,889 | 20 | 6 | 6 | 4 |
| HO1 | Horse | 10/3/05 | East Washington | 2,222,222 | 20 | 6 | 6 | 5 |
| HO2 | Horse | 10/3/05 | East Washington | 1,556 | 20 | 6 | 6 | 5 |
| DA1 | Cow | 10/3/05 | East Washington | 122,222 | 20 | 6 | 6 | 6 |
| DU1 | Duck | 10/3/05 | East Washington | 4,444 | 20 | 6 | 6 | 4 |
| GO1 | Goat | 10/3/05 | East Washington | 488,889 | 20 | 6 | 6 | 4 |
| | | | | **Totals:** | 200 | 60 | 60 | 51 |

## Source Species Identification

There were 75 isolates from water samples collected at the 3 sites that were analyzed using the RiboPrinter®, all of which yielded results confirmed by biochemical tests as *E. coli*. The ribopatterns contained 7-13 bands. Banding patterns for water sample and source species isolates were considered to be the same if there was 90% or greater similarity, except for the inclusion of the two water-isolate patterns that matched at 89%. Initial analysis using only the local database resulted in 47 source species identifications, or 63% of the 75 isolates. The New Hampshire database included all of the local database patterns and also had more species and overall patterns. Further analyses using the New Hampshire database resulted in even more source species identifications. All results presented are for analyses where the New Hampshire database was used to improve the results found with the local database.

Overall, sources for 55, or 73% of the 75 isolates were identified (Table 6). Thus, the results using a threshold of 90% as used in previous studies (Jones, 2004; Jones and Landry, 2004) provided a good balance between accuracy and isolate identification.

**Table 6. Ribotyping success (≥90% similarity) for *E. coli* isolates from three freshwater beaches.**

| Beach location | Sample site designation | Sample date | *E. coli* conc. cfu/100 ml | Total # isolates | Identified isolates | Unidentified isolates |
|---|---|---|---|---|---|---|
| Nottingham | NOTLF | 7/21/05 | 128 | 5 | 2 | 3 |
| | | 8/15/05 | 52 | 5 | 3 | 2 |
| | NOTCR | 7/6/05 | 8000 | 5 | 4 | 1 |
| | NOTRT | 8/29/05 | 144 | 5 | 2 | 3 |
| | | | TOTAL | 20 | 11 | 9 |
| Troy | TROLF | 7/21/05 | 40 | 5 | 1 | 4 |
| | | 8/3/05 | 36 | 5 | 5 | 0 |
| | | 8/30/05 | 72 | 5 | 5 | 0 |
| | TROCR | 7/5/05 | 68 | 5 | 3 | 2 |
| | | 8/18/05 | 420 | 5 | 5 | 0 |
| | | | TOTAL | 25 | 19 | 6 |
| E. Washington | WASLF | 7/21/05 | 128 | 5 | 3 | 2 |
| | | 8/3/05 | 6400 | 5 | 4 | 1 |
| | WASRT | 7/21/05 | 92 | 5 | 5 | 0 |
| | | 8/18/05 | 196 | 5 | 3 | 2 |
| | | 8/30/05 | 398 | 5 | 5 | 0 |
| | | 9/21/05 | 80 | 5 | 5 | 0 |
| | | | TOTAL | 30 | 25 | 5 |
| | | | **TOTALS** | 75 | 55 | 20 |

There were 12 (16%) of the isolates that matched database patterns at <90% similarities and were thus considered to be from unknown sources. These "unknown" source isolates may be from source species that were not included in the database, or from included species that lacked enough diversity of ribopatterns in the database to provide an identification of adequate accuracy.

There were also 8 (11%) isolates with ribopatterns matching database patterns shared by multiple, unrelated species. These were categorized as "mixed" source species, considered successful identifications but included in the "unknown" category. There are several reasons this may occur. Some *E. coli* strains may be adaptable to multiple types of environments and be common strains in numerous different source species. Alternatively, some strains found in fecal material from different source species may be transient strains that are only there for a relatively short period of time. The mechanism of introduction could be ingestion and digestion of prey organisms, exposure to the feces of other species at landfills or sewage treatment facilities, or even coexistence of multiple

species in the same area, like pets and humans or wild animals with overlapping habitats. In the end, the existence of different strains with the same profile can also imply that ribotyping with a single restriction enzyme may give inadequate detail to differentiate all strains. One alternative strategy is the use of a second restriction enzyme in the digestion of *E. coli* DNA that cuts the chromosomal DNA at different sites. The additional information that is provided by using two profiles for each *E. coli* isolate has greatly reduced this problem and made ribotyping more useful (Jenkins et al. 2003, Hartel et al. 2002, Samadpour 2002), although it is a more expensive overall procedure.

Overall, there were 12 different source species identified, including all those sampled from the local study areas (Table 7). Two other categories were also included as successful identifications, mixed avian (local duck & goose) and mixed wildlife. The most commonly identified source species was geese (17 isolates), followed by cows and mixed avian (7) sheep (6), horses and ducks (3), septage, goat, wastewater effluent and dog (2), with single isolates identified as coming from deer, red foxes, wild turkeys and mixed wildlife.

**Table 7. Source species for water sample *E. coli* isolates identified by ribotyping at three NH beaches.**

| | | | | | | **Local database** | | | | | | | | **NH database** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Birds | | | Livestock | | | | Human | Birds | Wild animals | | | Pet | Human |
| Beach location | Sample site designation | Sample date | *E. coli* conc. cfu/100 ml | Total isolates | Identified isolates | Duck | Geese | Mixed avian | Cow | Goat | Horse | Sheep | Septage | Wild turkey | Deer | Red fox | Mixed wildlife | Dog | Wastewater |
| Nottingham | **NOTLF** | 7/21/05 | 128 | 5 | 2 | | | 1 | 1 | | | | | | | | | | |
| | | 8/15/05 | 52 | 5 | 3 | | | 2 | | | | | | | | | | | 1 |
| | **NOTCR** | 7/6/05 | 8000 | 5 | 4 | | | | 2 | | | 1 | | | | 1 | | | |
| | **NOTRT** | 8/29/05 | 144 | 5 | 2 | | | | | | | | 1 | | | | 1 | | |
| | | | TOTAL | 20 | 11 | | | 3 | 3 | | | 1 | 1 | | | 1 | 1 | | 1 |
| Troy | **TROLF** | 7/21/05 | 40 | 5 | 1 | | | | 1 | | | | | | | | | | |
| | | 8/3/05 | 36 | 5 | 5 | | 3 | | | | | 2 | | | | | | | |
| | | 8/30/05 | 72 | 5 | 5 | | 4 | | | | | 1 | | | | | | | |
| | **TROCR** | 7/5/05 | 68 | 5 | 3 | | 2 | | | | | | | | | | | 1 | |
| | | 8/18/05 | 420 | 5 | 5 | | 4 | | | | | | | | | | | 1 | |
| | | | TOTAL | 25 | 19 | | 13 | | 1 | | | 3 | | | | | | 2 | |
| E. Washington | **WASLF** | 7/21/05 | 128 | 5 | 3 | | 2 | | | | 1 | | | | | | | | |
| | | 8/3/05 | 6400 | 5 | 4 | 1 | | | 1 | | 1 | | | | | | | | 1 |
| | **WASRT** | 7/21/05 | 92 | 5 | 5 | | | | 1 | | 1 | 2 | | | 1 | | | | |
| | | 8/18/05 | 196 | 5 | 3 | | 1 | 2 | | | | | | | | | | | |
| | | 8/30/05 | 398 | 5 | 5 | 1 | | 2 | | 2 | | | | | | | | | |
| | | 9/21/05 | 80 | 5 | 5 | 1 | 1 | | 1 | | | | 1 | 1 | | | | | |
| | | | TOTAL | 30 | 25 | 3 | 4 | 4 | 3 | 2 | 3 | 2 | 1 | 1 | 1 | | | | 1 |
| | | | **TOTALS** | 75 | 55 | 3 | 17 | 7 | 7 | 2 | 3 | 6 | 2 | 1 | 1 | 1 | 1 | 2 | 2 |

The percentage of isolates for which source species were successfully identified was 55% (11/20 isolates) in Nottingham, 76% (19/25 isolates) in Troy and 83% (25/30 isolates) in E. Washington (Table 6). There were 17/20 (85%) unique ribopatterns for water sample isolates from Nottingham, 17/25 (68%) from Troy and 24/30 (80%) from E. Washington. Overall there were 52/75 (69%) unique ribopatterns from all three beaches. There was one ribopattern that was common to 9 isolates from all three beaches, and three other patterns that were shared by 2-3 isolates from two beaches. The lower level of diversity (68%) of patterns at the Troy beach reflected water isolates from geese, and to a lesser extent from sheep, that had identical patterns and occurred on more than one sample date.

The number of different species identified as sources at each site was seven in Nottingham, eleven in E. Washington and only four in Troy. The number of isolates identified for each source species was relatively even for Nottingham and E. Washington, but was dominated by geese (13/19 isolates) at Troy. Sheep and cows were identified as sources at all three beaches, while horses, ducks, goats, deer and wild turkey were only identified at E. Washington, red fox and mixed wildlife only at Nottingham and dog only at Troy. The prevalence of geese at the beach in Troy may be related to the high *E. coli* concentrations in geese feces (Table 5) and the fact that, along with ducks, they often deposit feces directly into lakewater.

The identified source species for the water samples containing high levels ($\geq$ 6400 cfu/100 ml) of *E. coli* were similar in that at least half of the four identified isolates for each sample were livestock. At Nottingham on 7/6/05, the four identified isolates included 1 sheep, 2 cow and 1 red fox isolate. At E. Washington on 8/3/05, the identified isolates included 1 horse, 1 cow, 1 duck and 1 wastewater/human isolate. There was no dominant single source for either contamination event.

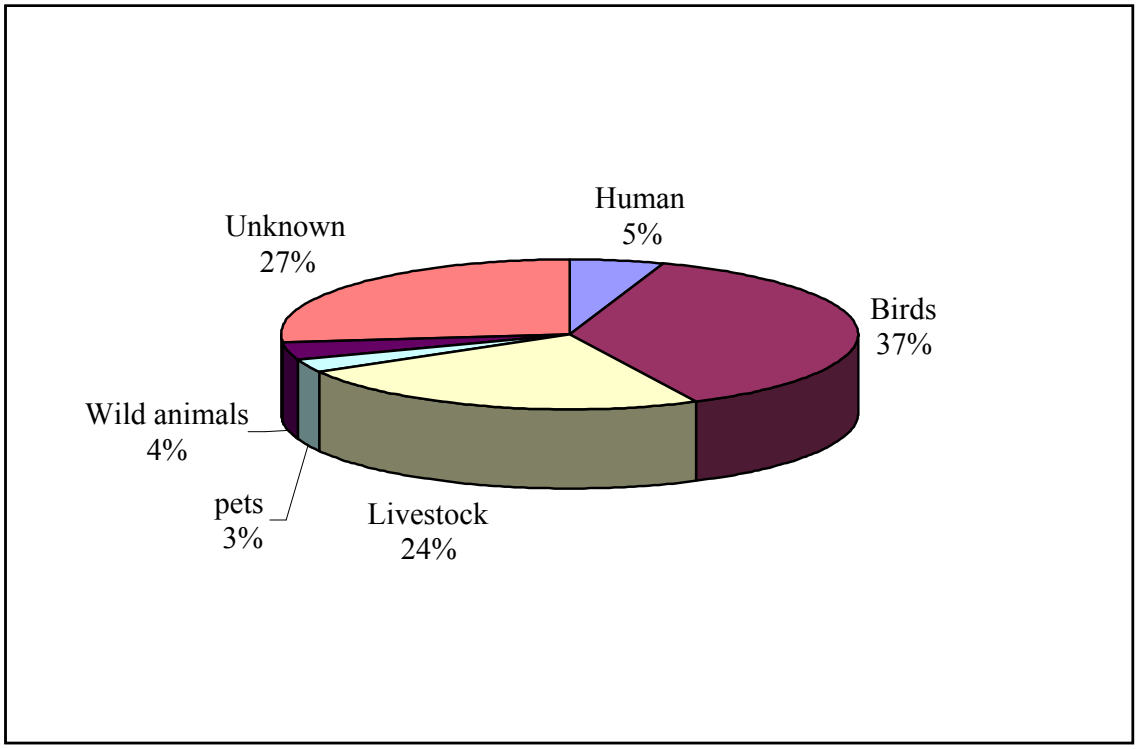
## Types of Identified Source Species

Any management actions taken in response to the results of this study would hinge on what types of source species were deemed significant sources of pollution. Because of this, a useful approach for analyzing results is to group source species into types that would trigger different management actions. The different types include humans, pets, domestic animals/livestock, wild animals and birds (Table 2). Overall, birds were the most prevalent (37%) source species type, followed by livestock (24%), humans (5%), wild animals (4%) and pets (3%) (Table 8, Figure 1).

**Table 8. Identified source species types for *E. coli* from three NH beaches.**

| Source type | Overall study # isolates | | Nottingham # isolates | | Troy # isolates | | E. Washington # isolates | |
|---|---|---|---|---|---|---|---|---|
| Human | 4 | 5% | 2 | 10% | 0 | 0% | 2 | 7% |
| Birds | 28 | 37% | 3 | 15% | 13 | 52% | 12 | 40% |
| Livestock | 18 | 24% | 4 | 20% | 4 | 16% | 10 | 33% |
| pets | 2 | 3% | 0 | 0% | 2 | 8% | 0 | 0% |
| Wild anim | 3 | 4% | 2 | 10% | 0 | 0% | 1 | 3% |
| Identified | 55 | 73% | 11 | 55% | 19 | 76% | 25 | 83% |
| Unknown | 20 | 27% | 9 | 45% | 6 | 24% | 5 | 17% |

**Figure 1. Identified source species for *E. coli* at three NH beaches.**




Human, wild animal and pet source isolates were only detected at low levels, and appear to have been insignificant sources of contamination at the beaches on the sample dates. Birds (geese) were the most significant source at Troy, and were equally significant as livestock at Nottingham and E. Washington. This profile of birds and livestock being the most significant types of source species differs from most other MST studies conducted in the NH Seacoast area. A more common profile of wild animals and humans as the most prevalent source species and pets, birds and domestic animals being of lower significance has been observed in other (coastal) MST studies (Jones and Landry 2003 & 2004, Jones et al. 2004b).

## Conclusions

The local source species database was invaluable for identifying source species. The majority of isolates could be assigned source species using the local database alone, while the NH database helped to augment source species identifications for species not included in the local database.

The overall level of detection (73%) was and excellent result. In other ribotyping studies conducted in NH, lower levels of identification have been observed. The EPA MST Guide Document (USEPA 2005) cites results from an *E. coli* ribotyping study in Virginia where 65% of isolates were identified to source species.

The level of detection varied for the different beaches, with the lowest at Nottingham (55% and the highest at E. Washington (83%).

The high level of identification at the E. Washington beach is important because of the consistent occurrence of *E. coli* levels that exceeded the state standard. A high level of identification provides a more accurate basis for interpreting the results.

To some degree, the number of source species and isolates in a local database from each beach could have influenced the degree to which the database could yield source species identifications. For this study, the most likely source species were chosen for inclusion in the local database. Only geese scat was collected at the Troy beach, only geese scat and septage samples were collected at the Nottingham beach, while fecal samples from five species were collected at E. Washington. To a large degree the results for the Troy and E. Washington beaches reflected the source species collected from those areas. The Nottingham beach results were less related to source species from that area. Use of the NH database with a much greater number of source species helped to provide identifications for more isolates. However, the actual sources, especially for isolates that could not be identified (which constituted a higher percentage of isolates compared to the other two beaches), may in part include sources not identified by this study.

These results suggest that the most prevalent types of source species are different at the three beaches and thus management strategies would also need to be different. A useful analytical strategy is to regard human, pet and domestic animal isolates as derived from human-related sources, while birds and wild animals probably originate solely from non-human related sources. In this regard, non-human related sources slightly outnumber human-related sources at Nottingham and E. Washington. The reduction or elimination of human sources could still provide a significant level of improvement in water quality to these sites. However, non-human related sources were twice as prevalent at Troy, so other strategies to deal with the geese may be needed.

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